suggest a dominant inheritance pattern. Interestingly, in contrast with other mutations, the R1459C mutation is localized extremely close to the carboxy-terminal end of the ABCC6 protein and leaves all functional sites of the protein apparently intact.

Thirdly, if pseudodominance explains all cases of putative dominant inheritance in PXE (up to 10%), the carrier frequency of PXE must be much higher than previously anticipated. This conclusion seems to be supported by recent findings: calculations on the basis of mutation analysis in ABCC6 suggest a heterozygote frequency for PXE of 1.25%–3.0% (Chassaing *et al.*, 2005). If this theoretical figure is correct, then the number of (homozygous) PXE patients actually observed in populations is unexpectedly low, if they are in Hardy–Weinberg equilibrium. Chassaing *et al*. (2005) suggested that one explanation could be that a number of sequence variations in the *ABCC6* gene would not lead to PXE at all. Indeed, the putative functional role of "neutral" ABCC6 polymorphisms, if any, has yet to be established. A number of so-called neutral variants may contribute to *ABCC6* function, dysfunction, and pathogenicity, especially in combination with more "severe" mutations. The existence of "mild" and "severe" pathogenic variants was also previously suggested for the ABCC6 family member ABCC7 (Welsh and Smith, 1993) and ABCR (Yatsenko *et al.*, 2001). On the other hand, this hypothesis was previously tested and rejected for ABCC6 in families from The Netherlands, because of high intrafamiliar variation in the PXE phenotype (Hu *et al.*, 2003).

Fourth, thoroughly investigated multigeneration pedigrees in human genetics are rare, which may partly explain why most PXE families are only described in two generations.

Finally, the influence of diet and other environmental factors on the PXE phenotype in patients and carriers is still poorly understood.

In summary, within the limitations given above, the current hypothesis is that PXE is an autosomal recessive disease. The recent construction of PXE mouse models, in which targeted loss of function of *abcc6* causes PXE-like symptoms, further supports that notion (Gorgels *et al.*, 2005; Klement *et al.*, 2005). These conclusions and findings probably mark the end of the autosomal dominant PXE segregation myth.

CONFLICT OF INTEREST

The author states no conflict of interest.

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Langerhans Cells on Guard in the Epidermis: Poised to dSEARCH and . . .?

Mark C. Udey[1]

Langerhans cells have long been considered to be prototypic immature dendritic cells. Results of experiments involving genetically engineered mice provide surprising new insights into Langerhans cell function *in vivo*. Nishibu and colleagues illustrate how these approaches can be used to visualize Langerhans cells *in vivo* in real time, and to assess aspects of their behavior in unperturbed skin and after activation.

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Although immune responses involve entire organisms, studies of immunity during the past several decades have emphasized, and perhaps overemphasized, reductionist *in vitro* experiments. Recently, the pendulum has swung back, and publication of novel results of *in vitro* experiments in high-profile journals essentially requires that authors demonstrate that their results are physiologically relevant. Although the bar has clearly moved up in this regard, the facility with which unambiguous *in vivo* experiments can be conducted and interpreted has also increased considerably.

The increased ease with which informative *in vivo* experiments can be carried out reflects increases in the knowl-

[1]*Dermatology Branch, Center for Cancer Research, National Cancer Institute, National Institutes of Health (NIH), Bethesda, Maryland, USA*

Correspondence: Dr Mark C. Udey, Dermatology Branch, Center for Cancer Research, National Cancer Institute, NIH, Building 10, Room 12N238, Bethesda, Maryland 20892-1908, USA.
Email: udey@helix.nih.gov

edge bases of immunology and genetics, as well as specific methodologic and technologic advances that have occurred in parallel. Development of the ability to manipulate the mouse genome almost at will has been critical. Genes of interest (such as those encoding cytokines) can now be expressed in a geographically and temporally restricted fashion in transgenic mice with the use of inducible or constitutively active, lineage- or tissue-selective promoters. Alternatively, gene "knock-in" approaches can be used to insert genes of interest downstream of endogenous regulatory regions, anticipating that maximum fidelity of expression will be achieved. Applications of transgenic mouse technology allow cells to be selectively "marked" with readily detectable proteins such as β-galactosidase or enhanced green fluorescent protein (EGFP), and even selectively targeted for destruction. Finally, genes of interest can be selectively and constitutively knocked out in mice by disruption of genes in embryonic stem cells via homologous recombination, or deleted in postnatal life using inducible promoters and Cre-*lox* technology.

The availability of increasingly sophisticated, minimally perturbing, intravital microscopic imaging technologies that allow observation of cell behaviors *in situ* has also been incredibly important (Halin *et al.*, 2005; Germain *et al.*, 2005). Successful and informative visualization of leukocyte–endothelial cell interactions in superficial or exteriorized blood vessels spurred interest in developing additional instrumentation that would permit leukocytes in other tissues to be studied. Using confocal and/or multiphoton fluorescence microscopy, it is now possible to image appropriately labeled cells in the subcapsular regions of lymph nodes and in thin tissues such as corneal epithelia and skin. Both tissue explants and vital tissues have been studied using these approaches.

Because of their importance and their essential roles in initiating and propagating T cell-dependent immune responses, dendritic cells (DCs) (including epidermal Langerhans cells (LCs)) are among the cells that have been actively studied by these recently developed *in vivo* approaches. DCs have been successfully imaged in lymph nodes, and the dynamics of DC–T cell interactions have been documented in real time (Germain and Jenkins, 2004). The recent identification of the C-type lectin Langerin as an LC-selective marker and characterization of the Langerin gene have afforded scientists the opportunity to study LCs *in situ* and to begin to definitively elucidate their function(s) (Valladeau and Saeland, 2005). Although prototypic in the sense that they have been studied more extensively than other DCs, LCs appear to represent a sublineage of DCs that are distinct from both interstitial and plasmacytoid DCs. Absolute dependence on transforming growth factor-β_1 for development and/or localization, the ability to persist in normal epidermis for months at a time, and derivation from slowly cycling local precursors are properties that differentiate LCs from other DCs, including interstitial DCs in the dermis (Valladeau and Saeland, 2005). It has been difficult to assign unique functional activities to LCs as compared with dermal DCs, however.

dSEARCH (dendrite surveillance extension and retraction cycling habitude) connotes the regular extension of LC dendrites between keratinocytes.

Recently, two groups of investigators independently created strains of mice in which complementary DNAs encoding closely related diptheria toxin receptor–EGFP fusion proteins were knocked into endogenous Langerin loci, albeit in slightly different positions and in different embryonic stem cells (Kissenpfennig *et al.*, 2005; Bennett *et al.*, 2005). Transgenic mice derived from these cells were viable and otherwise normal, and epidermal LCs uniformly expressed diphtheria toxin receptor–EGFP. Generation of corresponding Langerin–EGFP knock-in mice (Kissenpfennig *et al.*, 2005) allowed straightforward identification of LCs and related cells, as well as preliminary studies of LC behavior in explants and *in situ*, and tracking of LCs into lymphoid tissues after initiation of contact sensitivity responses. These investigations revealed that "resting" LCs are sessile and become motile after application of a strong inflammatory stimulus (for example, tape stripping) (Kissenpfennig *et al.*, 2005). Tracking studies revealed that, after application of a contact allergen, dermal DCs migrated to draining lymph nodes more quickly than epidermal LCs, and, somewhat surprisingly, that LCs and dermal DCs localized in adjacent but distinct regions of the T cell-rich paracortex (Kissenpfennig *et al.*, 2005).

A third group of investigators has generated a related transgenic mouse in which expression of diptheria toxin itself is driven by human Langerin upstream regulatory sequences (Kaplan *et al.*, 2005). In these mice, it is likely that LCs are deleted as soon as the Langerin promoter becomes active. This contrasts with mice described by Kissenpfennig *et al.* (2005) and Bennett and co-workers (2005), in which LCs are deleted only after systemic injection of diptheria toxin, a toxin that kills only cells that have been engineered to express the diptheria toxin receptor and does not perturb cells that are negative for this receptor. Systemic depletion of LCs began within hours after introduction of diptheria toxin, was virtually complete by 2 days, and lasted for several weeks or more (Kissenpfennig *et al.*, 2005; Bennett *et al.*, 2005).

Contact hypersensitivity (CHS) responses to allergenic small molecules such as trinitrochlorobenzene, oxazolone, and DNFB in mice have long been an experimental model of choice for *in vivo* studies of skin immune responses. Many papers have implicated LCs as key accessory cells in CHS responses, and direct presentation of hapten to naive T cells in draining lymph nodes by skin-derived LCs has been the preferred paradigm. Although it has been conjectured that LCs play similar roles in more complex skin immune responses, recent studies in murine models of cutaneous herpes simplex virus infection (Allan *et al.*, 2003) and leishmaniasis (Ritter *et al.*, 2004) suggest that DCs other than LCs may be directly involved in antigen presentation to effector T cells. The ability to study CHS responses in mice with isolated LC deficits has allowed a more rigorous assessment of the requirement for participation of LCs in this model. Studies of CHS reac-

tions in the three different LC-deficient mouse models reported to date have led to results that are simultaneously congruous and disparate.

Depletion of LCs did not abrogate CHS responses in any of the three strains studied. Whereas Kissenpfennig *et al.* (2005) reported that CHS responses were equivalent in LC-deficient and LC-replete mice, Bennett *et al.* (2005) noted that they were attenuated, and Kaplan *et al.* (2005) determined that they were enhanced. Because these studies were performed in different laboratories, the experimental procedures varied to some extent, and the differences reported were quantitatively modest, it is difficult to determine which result is "most correct." If the differences reported are confirmed in side-by-side comparisons using identical conditions and blinded observers, elucidation of the mechanism(s) responsible for the differences may provide important new insights into LC function. For the time being, however, at least we can say that LCs are not essential for CHS responses. This conclusion is very interesting in itself.

This issue of the *Journal* contains another elegant example of studies of epidermal LCs *in situ*. Transgenic mice and sophisticated imaging methods again feature prominently in the experiments. Akiko Nishibu, working with his colleagues in Akira Takashima's group at the University of Texas Southwestern Medical Center, has systematically catalogued the behavior of murine LCs in skin explants and *in vivo*, both in the presence and in the absence of inflammatory signals (tumor necrosis factor-α or contact allergen administration) that activate LCs and mobilize them from skin to draining lymph nodes (Nishibu *et al.*, 2006). Nishibu *et al.* used mice in which a complementary DNA encoding EGFP was knocked into the I-Aβ chain locus. Although I-Aβ is not a specific marker for LCs, in normal or acutely inflamed epidermis essentially all I-Aβ-expressing cells are LCs. Using time-lapse confocal microscopy and observation periods extending for several hours, the investigators could assess the location of cells in four dimensions, and locations of LC dendrites and cell bodies could be followed with relative ease (Nishibu *et al.*, 2006).

The paper by Nishibu *et al.* confirms that resting LCs are sessile and that application of inflammatory stimuli (explanting of skin or injection of tumor necrosis factor-α) induces both lateral and vertical motility of cell bodies, as reported by Kissenpfennig *et al.* (2005), and ultimately exit from the epidermis. Nishibu and co-workers also document, for the first time, an LC behavior that they term "dSEARCH" (dendrite surveillance extension and retraction cycling habitude) (Nishibu *et al.*, 2006). dSEARCH connotes the regular extension of LC dendrites between keratinocytes. In the absence of perturbation, only 5%–10% of epidermal LCs engage in dSEARCH. However, dSEARCH activity increases dramatically with regard to the frequency with which dendrites are extended and retracted, and the amplitude of the projections in tissue explants or in skin that has been injected with tumor necrosis factor-α or painted with DNFB. At this juncture, it is not possible to determine whether the LCs that exhibited dSEARCH without provocation were more "mature" than the others (perhaps because they had been in residence in the epidermis for the longest period of time) or if they were perhaps responding to subclinical inflammation.

Many of us who have looked at LCs in epidermal sheets using conventional immunofluorescence microscopy have wondered whether dendrites of adjacent LCs abut each other. This question is addressed directly by Nishibu *et al.* (2006). They report that although contact between adjacent resting LCs was rarely observed, contact between LCs was readily detected after application of maturation stimuli. These observations, when considered in the aggregate, suggest that LCs do indeed function as epidermal sentinels, surveying their environment and possibly communicating with each other by "touching" after activation. In light of the somewhat surprising results of the CHS experiments in LC knockout mice (Kaplan *et al.*, 2005; Kissenpfennig *et al.*, 2005; Bennett *et al.*, 2005), we are left wondering what these LCs may be telling each other, and possibly rethinking the roles that they play in skin-centered immune responses.

Although recent experiments leave us with many questions, several things are very clear. First, increased understanding of LC function *in vivo* will come only from additional *in vivo* experiments. Second, the various LC-deficient mice that have been generated will be invaluable in these experiments. Finally, new experimental systems and more sophisticated imaging technology may be required. At the conclusion of the recent 9th International Workshop on Langerhans Cells, organized by Niki Romani and Georg Stingl and held in Madeira, Ralph Steinman predicted that, in light of emerging new information, the next LC meeting, in Bern in 2007, will be very interesting. I agree with Dr. Steinman, and I am already looking forward to that meeting.

CONFLICT OF INTEREST

The author states no conflict of interest.

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